

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

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Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN 10/045,596 WITH ENTRY
OF THIS AMENDMENT

In the specification:

[0002] We next examined the possible sedimentation of the serum calcium phosphate complex during centrifugation, a property that might be anticipated for the complex based on the fact that calcium phosphate mineral phases typically have densities about 3 fold greater than serum. As shown in Table II, centrifugation of serum from rats treated with the 32mg dose of etidronate resulted in a pellet containing calcium, phosphate, and MGP. When the pellet was dissolved in acid and analyzed by SDS-PAGE, a major band was found at 59 kDa which accounted for at least 80% of the Coomassie staining (Figure **Error! Reference source not found.**). When this component was electrophoretically transferred to PVDF and subjected to N-terminal protein sequencing, one sequence was obtained, A-P-Q-G-A-G-L-G-F-R- (SEQ ID NO:[]1), which matches the N-terminal sequence of rat fetuin (Ohnishi *et al.* (1993) *J. Bone and Mineral Res.* 8: 367-377). The other major band in the gel had an apparent molecular weight of 66 kDa and accounted for about 10% of the total Coomassie staining; this band was identified as rat serum albumin by N-terminal sequence analysis. Based on the recovery of fetuin in the pellet, we estimate the weight ratio of fetuin to mineral phosphate in the pellet to be 3.4 mg/mg. Since the supernatant level of calcium and phosphate remained above the level in control serum (Table II), it is likely that centrifugation did not sediment all of the calcium complex in these experiments.